TEXAS AGRICULTURAL EXPERIMENT STATION

A. B. CONNER, DIRECTOR,
College Station, Texas

BULLETIN NO. 637 — AUGUST, 1943

THE CAROTENE CONSUMING POWER OF CERTAIN FEEDS

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B42 943-4500-L180

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SYNOPSIS

Preliminary observations indicated that some animal products had the power of destroying carotene and that such products of high carotene consuming power might injuriously affect chickens if included in the ration. Conditions which affect the carotene consuming power were studied. Methods for determining it were worked out and are given in detail. Samples of meat and bone scraps, meat scraps, tankage, dried fish, dried skim milk and dried butter milk may have a high consuming power for carotene. Vegetable feeds such as corn meal, cottonseed meal, wheat gray shorts and wheat bran very rarely if ever have any power to destroy carotene. The carotene consuming power is not constant but may increase or decrease from time to time. It is removed by autoclaving, but in as short a period as a week the feed may again have a high carotene consuming power. When fed to rats with feed containing moderate quantities of carotene, meat meal with high carotene consuming power sometimes decreased the vitamin A stored in the liver and sometimes did not. Fed to rats receiving very low quantities of carotene, meat meal with high carotene consuming power slightly decreased the length of life and the maximum weight attained. Fed to chickens, in 4 of 6 experiments the high carotene consuming power of meat products had no injurious effects. In the other two experiments, in one of which the ration contained low quantities of carotene, the by-product with high carotene consuming power appeared to cause vitamin A deficiency. The high carotene consuming power is injurious to chickens only under exceptional conditions, and when a ration very low in carotene or vitamin A is being fed.

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THE CAROTENE CONSUMING POWER OF CERTAIN FEEDS

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Occasionally samples of meat scraps, tankage, and fish meal in the diet of chickens have been reported to have a deleterious effect. Kaupp (6) noted that fish meals with a high free fatty acid content had an adverse effect, and Te Hennepe (quoted by Schroeder et al) 12 reported that meat scraps with a high free fatty acid content apparently reduced the egg production. Schroeder, Redding and Hurber (12) found that chickens fed meat scraps with a high free fatty acid value developed signs of vitamin A and D deficiency; the meat scraps after extraction with ether showed only a partial loss of the ability to produce the vitamin deficiencies while the extracted fat, when added to wholesome meat scraps, was not deleterious. Gray and Robinson (4) did not confirm these results. Sherwood, in the Division of Poultry Science of the Texas Agricultural Experiment Station, found that some samples of meat and bone scraps produced pronounced vitamin A deficiency. Lease, Lease, Weber, and Steenbock (7) found that when rancid fat or fatty acid peroxides were fed to rats at the same time with vitamin A or carotene there was a marked decrease in the vitamin A stored in the liver. Quackenbush, Cox and Steenbock reported (9) that the presence of alpha-tocopherol or catechol protected the carotene from the rancid fats. Harrellson et al (5) reported that fats which had been heated destroyed the vitamin A potency of other foods fed with them.

When meat products are moistened with a solution of carotene in petroleum ether, the petroleum ether allowed to evaporate and the mixture allowed to stand over night, there is sometimes a loss of carotene due to the presence of the meat product.

The conditions which affect this loss have been studied and a method derived for determining the carotene-consuming power of feeds.

Carotene oxidase which destroys carotene as reported by Sumner and Dounce (14) and Sumner and Sumner (14) to be present in the water soluble extracts of legumes, is different from the compounds in meat products which destroy carotene. A method for the determination of units of carotene oxidase in legumes has been reported by Reiser and Fraps (10).

EXPERIMENTAL ON METHOD

Preliminary experimental work was necessary in order to develop the method for determining carotene consuming power described below. Alfalfa leaf meal was not a suitable source of carotene, since destruction in it was both slight and irregular. In the procedure finally adopted a solution of purified carotene in petroleum ether was added to the sample, the petroleum ether allowed to evaporate and, after a period of time, the carotene that remained was purified and determined by means of a photoelectric colorimeter. The purification was found to be necessary because the petroleum ether usually dissolves colored material from the meat products. An equal amount of the carotene solution treated in the same way but without a sample was used as a standard. Some materials absorb carotene instead of destroying it. Other factors were also found to influence the results and must be carefully controlled in order to secure reliable results.

When the carotene solution was added to the meat product in an Erlenmeyer flask, higher losses occurred when the flask was unstoppered than when it was stoppered (Table 1). The vapor of petroleum ether protected the carotene in the stoppered flask. No difference was observed (Table 2) due to the increase in exposed surface when the meat product was spread over the bottom of a 400 ml beaker as compared with a 150 ml beaker. The quantity of sample affected the results (Table 3), a smaller percentage of carotene being destroyed when 4 or 5 grams of sample was used than when 1 gram was used. The quantity of carotene used had some effect, since a larger percentage was destroyed (Table 4) when 550 micrograms was used with a 5 gram sample than when 116 micrograms was used. Thus, the greater the relative amount of carotene present, the greater both the absolute amount and percentage de-

Table 1. The effect of stoppered and unstoppered flasks on the loss of carotene in contact with feeds.

	Stoppered feed loss %	Unstoppered loss %
Dried skim milk	17	74
50% protein meat and bone scraps	32	11
50% protein meat and bone scraps	1	17
50% protein meat and bone scraps	45	68
50% protein feeding tankage with bone	14	48
50% protein meat and bone scraps	1	1
50% protein meat and bone meal	3	21
50% protein meat and bone scraps	14	40
50% protein meat and bone scraps	0	2
50% protein meat and bone scraps	50	52
60% protein meat meal digester tankage	68	89
50% protein meat and bone scraps	4	20

Table 2. The effect of area of surface exposed on the percentage of carotene destroyed.

Sample	Loss	
	150 cc beaker %	400 cc beaker %
Meat and bone scraps	16	22
Meat and bone scraps	45	47
Tankage	45	37
Tankage	40	38
Tankage	43	34
Tankage	36	34

Table 3. Effect of the quantity of sample on the percentage of carotene destroyed.

Sample	1 gm. %	4 gm. %	5 gm. %
Fish meal	28	15	--
Tankage	80	63	--
Tankage	52	43	31
Tankage	53	45	28
Meat and bone scraps	24	15	--
Meat and bone scraps	71	45	--
Tankage	46	40	--
Tankage	44	36	--

Table 4. Effect of amount of carotene on the percentage of carotene destroyed.

Sample	Grams of sample	Loss		
		116 micrograms carotene %	340 micrograms carotene %	550 micrograms carotene %
Tankage	1	52	56	56
Tankage	5	35	52	62
Tankage	1	50	72	82
Tankage	5	39	57	67

stroyed. The reason for this result is not apparent as one would expect the destruction to vary directly with the amount of sample.

Dried skim milk and meat products from which the fat had been extracted have the power of absorbing carotene, holding it from solution in petroleum ether, to the extent of 16 to 30 per cent of the carotene added. This source of error was avoided by dissolving the milk product in caustic potash or treating the meat product with alcohol so as to bring the absorbed carotene into solution.

The temperature affects the quantity of carotene destroyed; the data in table 5 shows appreciably greater destruction at 35° C than at 6° C.

Table 5. The effect of temperature on the loss of the carotene in contact with feeds.

Sample	Loss	
	35° C %	6° C %
Tankage	91	28
Meat and bone scraps	71	20
Tankage	80	40
Fish meal	50	13

Portions of a freshly prepared solution of carotene in petroleum ether may be evaporated in a beaker and allowed to stand over night with little of no loss of carotene. But, if the solution is stored a few days, especially if exposed to light, the carotene becomes activated, so that although there is little loss of color from the solution, there may be considerable losses of carotene when the solution is allowed to evaporate and stand over night. It is seen from Table 6 that the loss may be 28 to 70 per cent of the carotene. The loss was less when a brown bottle was used for storage than when a clear glass bottle was used.

The activated carotene in an old solution may be partly protected from loss by meat products. As shown by the results in Table 7, there is less loss when such a solution is added to the meat products than when it is placed in a beaker alone and allowed to stand over night.

Table 6. Losses of carotene when freshly prepared or when old solutions were used.

	Carotene in the solution			Loss on exposure to air in beaker over night		
Age of solution in days	0 days ppm	4 days ppm	14 days ppm	0 days %	4 days %	14 days %
Purified carotene No. 1 in brown bottle	1.30	1.26	1.20	2	36	28
Purified carotene No. 1 in clear bottle	1.26	1.16	1.13	0	45	58
Unpurified carotene No. 1 in brown bottle	1.09	1.08	1.03	2	38	30
Unpurified carotene No. 1 in clear bottle	1.06	1.03	0.96	0	49	70
Purified carotene No. 2 in brown bottle	1.28	1.32	1.32	2	12	34
Purified carotene No. 2 in clear bottle	1.24	1.24	1.24	6	45	48
Unpurified carotene No. 2 in brown bottle	1.24	1.18	1.17	5	20	57
Unpurified carotene No. 2 in clear bottle	1.09	1.28	1.16	5	34	56

Table 7. Effect of exposed and old carotene solution on carotene consuming power of animal by-products.

	Fresh solution loss %	Old solution: Exposed solution used as stated loss %	Old solution: Original solution used as stated loss %
Meat and bone scraps	18	0	36
Meat and bone scraps	71	50	78
Meat and bone scraps	14	0	28
Meat and bone scraps	31	0	38
Meat and bone scraps	76	60	79
Fish meal	25	0	28
Fish meal	40	0	42
Sardine meal	40	0	47
Sardine meal	32	0	36

METHOD OF DETERMINATION

After considering the various factors discussed above the method of determining the carotene-consuming power of feeds decided upon is as follows:

Carotene solution: Purify 0.1 gm. of SMA carotene by solution in chloroform and precipitation with methanol (1). Dissolve 50 mg. of purified carotene in 2 ml of chloroform and make up to 500 ml with petroleum ether. Store the solution in a brown bottle in the refrigerator. Test the solution once a week by pipetting 1 ml into a 150 ml beaker, allowing to stand over night in an incubator at 35° C, adding 100 ml of petroleum ether and determining the carotene. If when compared with 1 ml of the original solution made up to 100 ml the loss exceeds 5 per cent, a new carotene solution should be prepared.

Procedure for tankage, meat and bone scraps and fish meals, weigh 1 gm of the sample to be tested into a 150 ml beaker and add 1 ml of the carotene solution, being careful to moisten the entire sample. Place in an incubator at 37° C over night. For controls, pipette 1 ml of the carotene solution into each of three 150 ml beakers and place with the samples. Add 90 ml of petroleum ether to each beaker. Stir well with a glass rod and let stand 5 minutes. Stir again and allow to settle. Make up to 100 ml. Take 50 ml, add 2.5 gm activated magnesium carbonate (1) shake well and centrifuge. Transfer the solution to another bottle containing about 1 ml of 1 per cent hydrochloric acid to remove any suspended material, shake, centrifuge and determine the carotene. For a standard, pipette 1 ml of the carotene solution into a 100 ml volumetric flask and make up to volume. Store in the refrigerator. Calculate the carotene destroyed by comparison with the standard. If appreciable loss occurs in the controls, a fresh solution of carotene should be prepared and the work repeated.

Procedure for skim milk. Transfer 1 gram to a 150 ml beaker and add 1 ml of carotene solution. Allow to stand over night in an incubator at 37° C. Add 20 ml of a 2 per cent potassium hydroxide solution. Stir with a glass rod until completely dissolved. Transfer to a separatory funnel and wash the beaker with two 10 ml portions of 95 per cent alcohol and three 10 ml portions of ethyl ether. Add the washings to the solution in the separatory funnel. Shake. Allow to settle and add the ether layer to the original ether extract. Wash the combined ether extracts with 3 portions of 100 ml of water. Dry the ether layer in an Erlenmeyer flask with anhydrous sodium sulphate and make up to volume in a 100 ml volumetric flask. Purify with magnesium carbonate as directed above and determine the carotene.

Procedure for animal products from which the fat has been extracted. Treat 1 gm with carotene solution as directed above. In the morning add 5 ml of alcohol. Warm for a few minutes on the water bath, cool, and add 95 ml of ethyl ether. Mix well, allow to settle and determine the carotene in the supernatant solution.

Table 8. Changes of carotene consuming power during storage.

	Where stored	Start	2 weeks	4 weeks	6 weeks	8 weeks	12 weeks
		loss %	loss %	loss %	loss %	loss %	loss %
Meat and bone scraps	Laboratory	9	14	25	21	17	36
Meat and bone scraps	Laboratory	34	70	67	58	59	--
Meat and bone scraps	Laboratory	5	14	21	18	20	44
Meat and bone scraps	Laboratory	47	22	18	13	7	23
Meat and bone scraps	Laboratory	82	85	82	74	73	84
Fish meal	Laboratory	0	16	25	13	9	26
Fish meal	Laboratory	5	36	42	31	22	36
Dried skim milk	Laboratory	72	63	67	--	--	--
Sardine meal	Laboratory	3	41	50	33	32	--
Sardine meal	Laboratory	5	34	28	28	22	80
Dried buttermilk	Laboratory	0	14	21	--	--	--
Meat and bone scraps	Incubator 35° C	9	5	11	--	--	--
Meat and bone scraps	Incubator 35° C	34	27	11	--	--	--
Meat and bone scraps	Incubator 35° C	5	5	8	--	--	--
Meat and bone scraps	Incubator 35° C	47	3	5	--	--	--
Meat and bone scraps	Incubator 35° C	82	52	35	--	--	--
Fish meal	Incubator 35° C	0	14	18	--	--	--
Fish meal	Incubator 35° C	5	18	25	--	--	--
Dried skim milk	Incubator 35° C	72	29	46	--	--	--
Sardine meal	Incubator 35° C	3	27	28	--	--	--
Sardine meal	Incubator 35° C	5	25	28	--	--	--
Dried buttermilk	Incubator 35° C	0	0	8	--	--	--

FACTORS WHICH AFFECT CAROTENE CONSUMING POWER

A large number of feeds were tested for carotene consuming power by the methods described. It was found to occur almost entirely with meat products, dried milk products and dried fish. The carotene consuming power is not constant, so that the same sample may give different results from time to time. Table 8 shows some variations which occurred during storage under laboratory temperature and at 37° C. The carotene consuming power of some samples increased and that of others decreased.

Light may increase the carotene consuming power. Samples of meat and bone scraps were divided; one portion of each was placed in clear glass bottles and another portion wrapped in aluminum foil was placed in a similar bottle. After 4 weeks (Table 9), the samples exposed to light had appreciable higher carotene consuming power than those not exposed. As shown in Table 10, portions from the surface of samples in bottles, in some cases, had a higher carotene consuming power than portions taken from below the surface.

Autoclaving meat and fish products at 20 pounds pressure for one hour decreased the carotene-consuming power of most of the samples as shown in Table 11. The change was not permanent, as is shown in Table 12. After one week, the autoclave mples again had a high carotene-consuming power. When samples which had a high carotene consuming power were ashed, the ash had practically no carotene consuming power. Table 13. This showed that the ash constituents were not responsible for the consuming power.

Table 9. Effect of light on the sample on destruction of carotene by feeds.

	Start %	Exposed to light %	Wrapped in foil %
Meat and bone scraps	5	46	11
Meat and bone scraps	58	69	27
Meat and bone scraps	11	33	10
Fish meal	25	29	25
Tankage	48	75	50

Table 10. Effect of location of portion used in sample bottle on destruction of carotene.

	Surface sample %	Subsurface sample %
Meat and bone scraps	41	10
Meat and bone scraps	70	52
Meat and bone scraps	24	10
Fish meal	17	15
Fish meal	39	35

Table 11. Effect of autoclaving upon percentage of carotene destroyed.

Name of sample	Untreated %	Autoclaved 60 min. %
Meat and bone scraps	58	7
Tankage with bones	68	7
Meat and bone scraps	58	14
Meat and bone scraps	36	3
Meat and bone scraps	45	6
Sardine meal	16	0
Fish meal	47	1
Meat and bone scraps	78	40
Tankage	94	10
Raw bone meal	78	83
Fish meal	27	5
Meat and bone scraps	29	3
Meat and bone scraps	63	7
Tankage	25	12
Tankage	46	7
Tankage	42	20
Tankage	44	20

Table 12. Effect of storage 1 week after autoclaving upon percentage of carotene destroyed.

Name of sample	At beginning		After 1 week	
	Untreated %	Autoclaved %	Untreated %	Autoclaved %
Meat and bone scraps	35	17	--	67
Meat and bone scraps	59	38	78	64
Tankage	50	10	90	63
Meat and bone scraps	70	35	85	73
Tankage	63	34	87	79
Tankage	46	38	--	65
Tankage	59	28	85	66

Table 13. Carotene consuming power of the ash of meat products.

Name of sample	Loss of carotene	
	Untreated sample %	Ash %
Tankage	35	5
Meat and bone scraps	78	5
Tankage	45	9
Tankage	94	0
Tankage	70	0
Meat and bone scraps	8	0
Meat and bone scraps	9	3
Meat and bone scraps	0	0
Meat and bone scraps	7	0
Meat and bone scraps	5	0

Table 14 shows that the fat extracted from the feed by petroleum ether consumed carotene in some cases and did not in others while the residue from which the fat had been extracted consumed carotene in all cases. One gram of the feed was extracted six times on a filter paper with 10 cc of 30° to 60° boiling petroleum ether. The extract was collected in a 300 cc Erlenmeyer flask provided with a ground glass joint. The ether was removed by reduced pressure and 1 ml of the petroleum ether carotene solution added to the fat and the carotene consumed determined. The potency of the residue was determined at the same time. The results are given on Table 14. This experiment showed that most of the substance causing the destruction of carotene remained with the fraction insoluble in petroleum ether.

Table 14. Percentages of carotene destroyed by fats soluable in petroleum ether and extracted residues.

Name of sample	Fats soluble in petroleum ether loss %	Extracted residues %
Tankage	42	58
Meat and bone scraps	26	54
Meat and bone scraps	40	55
Meat and bone scraps	20	47
Meat and bone scraps	2	72
Dried skim milk	0	35
Meat and bone scraps	0	78
Fish meal	0	78
Meat and bone scraps	0	31
Fish meal	5	39

It was thought that the failure to extract the carotene destroying substance with petroleum ether might be due to the presence of fatty acid peroxides which are only slightly soluble in that solvent, but are soluble in alcohol and ethyl ether. To test this point a series of determinations were made by boiling 1 gram of the sample with 10 ml of redistilled alcohol for 10 minutes, adding 25 ml of peroxide-free ethyl ether while still warm, and filtering into a 300 ml Erlenmeyer flask provided with a ground glass joint. The extraction was repeated twice with 5 ml of alcohol and 15 ml of ether and finally with two 15 ml portions of ether only. The alcohol and ether was drawn off by means of reduced pressure and the carotene consuming power of the fat and of the extracted residues determined. It was found that the carotene is tenaciously absorbed on the fat-free material and must be removed by heating with alcohol. The procedure was modified to permit this manipulation. Table 15 shows the results of the determinations. Both the fat and the extracted residue had carotene consuming power which showed that the carotene consuming power was not due entirely to fatty peroxides.

Table 15. Percentage of carotene consumed by fat free residue and by the fat extracted from meat products.

Sample	Untreated	Fat-free residue	Fat
Meat and bone scraps	10	22	31
Meat and bone scraps	11	31	18
Meat and bone scraps	11	18	23
Meat and bone scraps	12	25	29
Meat and bone scraps	13	20	7
Meat and bone scraps	38	40	34
Meat and bone scraps	75	36	65
Tankage	70	41	70
Meat and bone scraps	78	42	50
Tankage	72	38	48
Tankage	59	31	54
Tankage	38	23	40

The effect of autoclaving upon the fat and the fat-free residue was studied. Four 1 gram samples of a series of meat products were extracted with alcohol and ether as in the method described above. Two fat-free and two fatty fractions were autoclaved. The carotene consuming power of one autoclaved and one untreated fraction of each material was determined at once. The corresponding fractions were allowed to stand one week before their potency was determined. The results of this experiment are tabulated on Table 16. Autoclaving destroyed some of the potency of both the fat free and fat fractions. The fat free residue did not increase in carotene consuming power in a week of storage but the carotene consuming power of both the autoclaved and the untreated fat increased to almost 100 per cent.

Table 16. Percentage of carotene consumed by fat and by fat-free residue of meat products before and after autoclaving.

Sample	Immediately				After one week			
	Fat-free residue		Fat		Fat-free residue		Fat	
	Auto-claved	Not auto-claved	Auto-claved	Not auto-claved	Auto-claved	Not auto-claved	Auto-claved	Not auto-claved
Meat and bone scraps	8	23	36	88	11	20	99	98
Tankage	10	33	35	94	12	32	98	97
Tankage	12	29	76	82	13	35	96	95
Tankage	11	37	62	84	15	40	97	96
Tankage	12	22	62	94	13	20	96	97

The instability of the active principle, when considered with work of others (7), seemed to indicate that peroxides of the fatty acid are responsible. This simple theory was very attractive, but had to be modified when it was found that repeated extractions with boiling alcohol and ether (Table 15) did not completely remove the carotene consuming power. There is the possibility that in the unextracted material only the fatty fraction is active but that after removal of the fat there is a new effect due to surface phenomena. We have seen above how meat scraps protected unstable carotene from oxidation by the air and that this protective power may reside in the fat fraction, as can be seen in Table 15. When meat scraps of low carotene consuming power were extracted, the fat-free fraction had considerably more potency than the unextracted material. In all cases the carotene destroying power of the extracted material plus that of the residue was considerably higher than that of the unextracted sample. Schroeder, Redding, and Huber (12) found that ether extracted meat scraps when fed to chickens produced the same signs of vitamin A and D deficiency as the unextracted material, indicating that there may be a substance there responsible for the effect rather than just a surface effect. Some premilinary experiments have shown that dried blood has a high carotene consuming power but no relation was found between the carotene consuming power of samples of meat scraps and their dried blood content.

EXPERIMENTS WITH RATS.

It was thought possible that meat by-products of high carotene consuming power when fed with feed containing carotene might destroy carotene in the digestive tract of animals and so reduce the quantity available for use. Such destruction was found to occur by Powick (8) and by Lease et al (7) when rancid fats or peroxides of fatty acids were fed to rats, resulting in decreased storage of vitamin A in the livers. A similar method of experiment was applied to meat products.

In order to ascertain whether the carotene consuming power was of practical importance, experiments were made to see if it would affect the quantity of vitamin A stored in the livers of rats, or the length of life and weight when fed when only small amounts of carotene were fed.

PROCEDURE AND RESULTS WITH LIVER STORAGE.

Rats 21 days old were selected from females fed on a diet low in vitamin A potency since the birth of their young, and divided into 2 groups, equalized as to sex and litter. One group received meat meal with a high carotene-consuming power, and the other a similar by-product with a low carotene consuming power. Both groups were fed a diet usually composed in per cent of white corn meal 37, wheat gray shorts 25, casein 12, yeast (irradiated) 3, salt 1, tri-calcium phosphate 1, calcium carbonate 1, meat meal to be tested 20 and alfalfa replacing corn meal to furnish 10 micrograms of carotene per gram, except as otherwise noted. After 14 days the rats were killed, and the livers removed and weighed. The spectro vitamin A was determined by means of a Bausch & Lomb medium spectograph by methods already described (3).

Ten rats per group were used in the first 3 comparisons and 6 per group in the others. The quantity of carotene consumed during each experiment was comparatively uniform, being from 1000 to 1100 micrograms.

Table 17 contains the result of 10 comparisons. In 5 of the 9 comparisons, in which alfalfa was used as the source of carotene the spectro vitamin A was lower in the livers when the high carotene consuming meat meal was fed than when the meat meal had a low carotene consuming power. With the remaining 4 comparisons, the high carotene consuming meat meal gave equally as good results as the low one. With cod liver oil as a source of vitamin A, in the tenth comparison, the spectro vitamin A in the liver of the rat receiving meat meal of high carotene consuming power was 89 per cent of that secured when the low consuming power product was used. Apparently the carotene consuming power of the meat meal interfered with the utilization of carotene by rats for liver storage in some cases and did not interfere in others. The effect in general was not great, so that the high carotene consuming power of meat meal does not seem to seriously affect the storage of vitamin A in the livers of rats.

It is to be noted from Table 17 that appreciably less spectro vitamin A was stored in June and July (35.5 and 48.1 micrograms per liver)

Table 17. Spectro vitamin A in micrograms per liver as affected by low and high carotene consuming power of meat meals.

Experiment number and source of vitamin A potency	Spectro vitamin A per liver		Percent high of low	Month of experiment
	Low gms.	High gms.		
Alfalfa				
3114	116.4	80.6	69	1940 November
3114	83.8	94.5	112	1940 December
3114	74.0	68.9	93	1941 February
3185	96.8	68.1	70	March
3185	63.5	57.3	90	May
3229	51.6	48.1	93	June
3235	36.3	35.5	98	July
3418 (no oil)	77.4	77.4	100	1942 May
3418 (10% oil)	91.5	110.4	121	May
Cod liver oil				
3179	212.6	190.3	89	March

than in the cooler months, as much as 80.6 in November and 94.5 micrograms in December.

Substitution of 10% cottonseed oil for 10% corn meal in the ration in another Experiment increased the storage of spectro vitamin A in the liver from 77.4 micrograms without oil to 110.4 micrograms per liver. The effect of some different quantities of meat meal and different quantities of carotene is shown in Table 18.

Table 18. Effect of percentage of meat meal in the ration on spectro vitamin A in rat livers.

	Spectro vitamin A per liver micrograms
Carotene 10 parts per million	
No meat meal	44.7
10% meat meal	46.2
20% meat meal	52.5
30% meat meal	43.0
Carotene 5 parts per million	
No meat meal	25.6
10% meat meal	34.3
20% meat meal	35.4
30% meat meal	35.3

In the experiment described above, the total storage of spectro vitamin A was compared. Treichler, Kemmerer, and Fraps (15) have shown that part of the spectro vitamin A is not vitamin A at all, since it may increase when the rat is fed upon a diet practically free from vitamin A. In one experiment the meat meals were also fed without carotene, with the results given in Table 19. The livers of the rats which did not receive carotene contained 22.7 and 25.1 micrograms of pseudo spectro vitamin A per liver. When correction was made for these quantities, the spectro-vitamin A stored from the meat meal with low carotene consuming power was 12.4 micrograms per liver, while that stored from the meat meal with high consuming power was 8.7 micrograms per liver.

The carotene consuming power of animal products in the diet may affect the storage of vitamin A in liver, but not to a great extent.

Table 19. Effect of meat meal alone and with carotene on spectro vitamin A in livers.

Addition and carotene consuming power of meat meal	Spectro vitamin A micrograms per liver	
	Total	Net*
No carotene—low	22.7	--
Carotene—low	36.3	12.4
No carotene—high	25.1	--
Carotene—high	35.1	8.1

*Corrected for difference in weight of livers.

Effect of Carotene Consuming Power on Longivity and Gain of White Rats.

The experiments on storage in livers were made on moderate levels of vitamin A. It was thought that the carotene consuming power might have a greater effect if low levels of carotene were fed.

At the time of weaning, litter mates were divided as evenly as possible between several groups and 4 males and 4 females were placed upon each feed containing 0.1 micrograms of carotene per gram, or its equivalent in other forms. The feed in percentage consisted of meat meal 20, heated corn starch 57, cottonseed oil 8, irradiated yeast 1, non irradiated yeast 9, salt mixture 4 and sodium chloride 1. It was planned to continue the experiment until all the rats died, but they lived much longer than expected, and those alive after 470 to 698 days were killed and the experiment discontinued.

The carotene in alfalfa was determined as pure carotene (1), the vitamin A in cod liver oil as spectro vitamin A (3) and reduced to carotene equivalent, and the pure carotene in yellow corn was calculated to carotene equivalent (2).

The results of the experiments in which 0.1 micrograms carotene per gram of feed was used, are given in Table 20. Average length of life and average maximum weight were both lower with the rats which received the meat meals with high carotene consuming power. The differences were not great, however, but on the other hand were comparatively small. Very little carotene was destroyed in the rats by the meat scraps with high carotene consuming power.

Table 20. Effect of carotene consuming power of meat meals on longevity and gain in weight of white rats.

Type of meat meal in ration	Source of vitamin A potency (0.1 micrograms per gram of ration)	No. rats on experiment	No. rats living when exp. closed	Maximum weight of rats (grams) Aver. 4 males	Aver. 4 females	Aver. males and females	Aver. length of life days
Low carotene consuming	alfalfa	4 F, 4 M	4 F, 1 M	336	212	274	519
High carotene consuming	alfalfa	4 M, 4 F	2 M, 3 F	301	192	247	502
High carotene consuming	cod liver oil	4 M, 4 F	1 M, 1 F	302	204	253	452
High carotene consuming	yellow corn	4 M, 4 F	1 M, 1 F	250	197	224	478

Experiments With Chickens.

This work was started to attempt to learn the cause of certain chick losses in Texas that appeared to be associated with certain animal protein feeds. When the feeding of the particular animal protein feed was discontinued, the losses usually stopped. It was reported that the chicks seemed to be suffering from vitamin A deficiency. The work of Schroeder (12) indicated that there was a rather wide variation in the free fatty acid content of different samples of meat scrap studied by him, and the work of Schroeder (12) as well as the work of Powick (8) indicated that vitamin A is progressively destroyed or inactivated by free fatty acids. It was thought that this trouble with chicks might be caused by a destruction of all or part of the carotene of vitamin A in the feed. In order to test this hypothesis, and to ascertain the importance of such carotene consuming power several experiments were made. Samples of meat scrap were secured directly from a number of manufacturers. Four samples of 50 per cent protein meat scrap and one sample of 55 per cent protein meat scrap were used in the first experiment. Nine per cent of the different lots of 50 per cent protein meat scrap and 8 per cent of the 55 per cent protein meat scrap were used in the respective rations. These were used in experiment 1, in which 175 micrograms of carotene in oil was used per 100 grams of ration, and in experiment 2 in which 5 pounds of dehydrated alfalfa leaf meal containing from 195 to 142 micrograms of carotene per gram was used per 100 pounds of feed. The rations were made up at two week intervals during the experiment which continued for 10 weeks. The rations used are given in Table 21.

Table 21. Percentage of ingredients of rations for experiment 1 and experiment 2.

	Experiment 1	Experiment 2
Ground kafir	40*	43
Ground milo	6	--
Wheat gray shorts	20	20
Ground barley	--	--
Ground whole oats	10	10
Dehydrated alfalfa leaf meal	--	5
Meat scraps	9*	9
41% protein soybean oil meal	4.5	4.5
43% protein cottonseed meal	4.5	4.5
Dried whey	3	1
Oyster shell	2	2
Salt	1	1

*47% kafir and 8% meat scraps were used in the rations containing 55% protein meat scraps.

According to Table 22, all of the chicks in lot 5 died before the chicks were 8 weeks old. In lot 10, which received the same meat scrap as lot 5, the mortality was 40 per cent even though the chicks received a very liberal supply of carotene from alfalfa leaf meal. With the chickens in both of these lots marked symptoms of vitamin A deficiency were noted. Although the chickens were on wire floors in battery brooders, a rather large number of the chicks in lot 5 also had coccidiosis. One case was noted in lot 10 and one in lot 4. This indicates the lack of resistance to infections of chicks deficient in vitamin A.

The free fatty acid content of the meat scrap which did not cause a loss was 7.8 per cent compared with 2.7 per cent for the sample with

Table 22.—Effect of meat scraps on mortality and gains in weight experiments 1 and 2.

	Experiment 1					Experiment 2				
Lot numbers	1	2	3	4	5	6	7	8	9	10
Carotene consuming power	0	--	--	--	58	0	--	--	--	58
Free fatty acid content of meat scrap	--	--	--	--	--	7.8	--	--	--	2.7
Mortality after first two weeks, per cent	8.7	23.1	23.1	32.0	100.0	0	0	0	0	40.0
Gains in weight of cockerels, gm.	588	568	402	473	--	599	667	526	510	347
Gains in weight of pullets, gm.	487	385	373	417	--	540	503	397	424	289
Mean gains in weight, gm.	538	477	388	445	--	570	585	462	467	318
Grams of feed per gram of gain	3.39	3.70	4.28	4.08	--	3.58	3.31	3.85	3.79	4.54

which the mortality was high. The free fatty acids do not account for the vitamin A deficiency. The carotene consuming power of the meat scrap that caused the greatest loss was 58 per cent as compared with 0 per cent for the meat scrap used in lots 1 and 6 which gave good results.

On January 2, 1941, Experiment 3 was started with twelve different samples of animal protein feeds ranging in carotene consuming power from 0 to 78 per cent. All contained 175 micrograms of carotene from alfalfa leaf meal per 100 grams of feed. The chicks were fed ten weeks. The mortality ranged from 0 to 29 per cent, but there was no correlation between gains in live weight or mortality and carotene consuming power as determined by the methods already mentioned. Neither was there any marked indications of vitamin A deficiency in any of the chicks.

On May 14, 1941, Experiment 4 was started in duplicate using samples of meat scraps with high and with low carotene consuming power; samples of fish meal with low carotene consuming power, and a sample of tankage with high carotene consuming power. No correlation was noted between gains in live weight or the mortality and the carotene consuming power of the animal protein feeds in this experiment. Very few birds showed symptoms of vitamin A deficiency. These chicks received 175 micrograms of carotene from alfalfa leaf meal per 100 grams of ration.

In the spring of 1942, twelve samples of meat scrap were found to have a carotene consuming power ranging from 1.4 to 97.8 per cent. On April 24, 1942, the two samples of meat scrap having the highest carotene consuming power and the two samples having the lowest consuming power were used in rations that were fed in quadruplicate, (Experiment 5). Two series of rations contained 125 micrograms of carotene per 100 grams of feed and two contained 200 micrograms. On both levels of carotene, one set of chicks received carotene in oil and the other set received carotene from alfalfa leaf meal.

In experiment 5 there were few birds showing symptoms of vitamin A deficency, and there was no correlation between the carotene consuming power as determined by the chemical method and the gain in live weight or the mortality. In fact, there were no significant differences in mortality. The gains in weight were lowest for ration 3 in all of the different series even though this ration was the second lowest in the carotene consuming power. Some factor other than carotene destruction must have caused this poorer gain.

In the fall of 1942, six samples of meat scraps and one sample of blood meal were secured. Experiment 6 was started on January 5, 1943 with rations for each group adjusted to contain the same levels of proteins, minerals and vitamins. They contained from 38.9 to 39.65% ground milo, 20% each of ground barley and wheat gray shorts, 4% each of soybean oil meal and cottonseed meal, ½% of dehydrated alfalfa leaf meal, 1% distillers' solubles, from 0.6 to 5.1% of minerals and from 6.25 to 10.0% of the animal protein feed being studied. They also con-

Table 23. Relation of carotene consuming power to mortality, gain in weight and vitamin A lesions of chickens.

Lot numbers	1	2	3	4	5	6	7
Carotene consuming power, per cent	45	54	34	16	32	0	32
Mortality after first 2 weeks, per cent	0.0	12.0	52.0	4.0	20.0	20.0	25.0
Gain in weight, cockerels, gm	797	774	623	762	661	809	689
Gain in weight, pullets, gm	655	560	471	726	525	705	701
Mean gain in weight, gm	726	667	547	744	593	757	695
Grams of feed per gram of gain	4.63	4.73	6.23	4.63	5.79	4.62	5.13
Per cent having vitamin A lesions after 8 weeks on experiment	4.0	40.9	50.0	12.0	17.4	13.0	45.0

tained approximately 100 micrograms of carotene, and 44 units of vitamin D per 100 grams and 6 grams of manganese sulphate per 100 pounds. As shown in Table 23, decidedly the poorest gains, greatest mortality, greatest percentage of chicks showing vitamin A deficiency at the end of the eighth week of the experiment and the poorest utilization of the feed were found with the ration containing blood meal fed to lot 3. This blood meal had a carotene consuming power as determined chemically of 34 per cent as compared with 45 and 54 per cent for the feeds used in rations 1 and 2 respectively. The chicks receiving ration 2 showed the third highest percentage of vitamin A deficiency. The gains in live weight after 12 weeks feeding were greatest and the incidence of vitamin A deficiency was lowest in the case of the chicks in lot 6, ration 6, but four chicks died from this pen. This diet contained the meat scrap which had no carotene consuming power according to chemical determination. The mortality and number of chicks showing vitamin A deficiency at the end of eight weeks of the experiment were low with the chicks in lot 4, which received a ration containing a meat scrap having a 16 per cent carotene consuming power. The weights of these chicks were very satisfactory.

DISCUSSION OF EXPERIMENTS ON CHICKENS.

Experiment 6 and Experiments 1 and 2 indicate that there is a definite depressing effect by some animal protein feeds on the carotene content of a ration. It appears that there may be some other factor involved other than that represented in the carotene consuming power. This work and the use of this carotene consuming power is further confused by the variability of the percentage of destruction by animal protein feeds as determined at different times. This is well shown in Tables 5, 8 and 24 that feeds that may at first have a low carotene consuming power but later have somewhat higher consuming power and feeds that have a very high consuming power may later have a consuming power as low as 30 per cent.

It is true that these factors of carotene destruction do not seem to be present in all animal protein feeds in sufficient amounts to cause trouble

Table 24. Variations in the carotene consuming power of certain feeds. (Per cent.)

Poultry analysis number	Consuming power				
	Dates Determined				
	1-9-41	5-7-41	6-3-41	7-2-41	7-29-41
950	0	3		9	13
944	78	64		50	30
940	63	8	11	8	17
949	76	24	70	60	54
948	20	32			
947	55	18			
946	5	1			
945	45	14			
943	0	13			
942	59	9			
941	55	31			
951	47				

with chicks, but the fact that they do occur in certain feeds of animal origin makes this a real problem for poultrymen who may be feeding rations rather low in carotene.

ACKNOWLEDGMENT

Dr. A. R. Kemmerer and other members of the staff made analyses of livers for spectro vitamin A and otherwise helped in the work. J. R. Couch and V. H. Melass of the Division of Poultry Husbandry also assisted in the work.

SUMMARY.

Certain animal by-products such as meat scraps, tankage, dried fish and dried milk have the power of destroying carotene. Vegetable feeds very seldom had any carotene destroying power.

The carotene-consuming power is affected by exposure to air, the quantity of the sample, the temperature and the quantity of carotene in relation to the quantity of the sample. Carotene solutions in petroleum ether became activated in a short time so that carotene is lost when the petroleum ether is allowed to evaporate and the mixture to stand over night. Animal by-products may decrease the loss of carotene in such solutions. Milk products may absorb carotene as well as destroy it.

A method of determining the carotene consuming power of feeds is given in detail. Carotene consuming power may change in storage, so that the same sample may give different results at different times. Samples high in carotene consuming power at first may decrease in this power, and those low in carotene destroying power may increase. Exposure to light may increase carotene consuming power. Autoclaving decreased carotene consuming power but the changes were not permenant, even for as short a period as one week.

Both the fat and residue from which the fat had been extracted had carotene consuming power.

The carotene consuming power may have been partly due to fatty acid peroxides, but not entirely. Materials subjected to treatment which should have removed fatty acid peroxides still had a high carotene consuming power.

When meat meal having high carotene consuming powers was fed to rats, in 5 of 9 experiments the livers contained less vitamin A than when similar feed having low carotene consuming power was fed. The differences, however, were comparatively small, and in 4 of the experiments the high carotene consuming power did not decrease the vitamin A stored. Rats fed on a mixture containing 0.1 micrograms of carotene per gram, with meat meal of high carotene consuming power had a slightly shorter average length of life and attained a lower average maximum weight than rats fed corresponding meat meal with low carotene consuming power. The differences were comparatively small.

Six experiments were made to ascertain the effect of carotene consuming power upon gains in weight and mortality on growing chickens. In 4 of the experiments, the carotene consuming power had no effect. In two of the experiments, the high carotene consuming power was detrimental to the chickens.

Feeds of high-carotene consuming power may sometimes cause injury to chickens through vitamin A deficiency if the ration is low in carotene or vitamin A potency. Otherwise a high carotene consuming power does not appear to be injurious.

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